

Investigation of Benzene-DNA Adducts and Their Detection in Human Bone Marrow

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We have examined DNA adduct formation in HL-60 cells and human bone marrow treated with either hydroquinone or *p*-benzoquinone and have found that these treatments produce the same DNA adduct in both cell types. The DNA adduct level from these treatments varied from 0.05 to 7.5 adducts per 10⁷ nucleotides as a function of treatment time and concentration for both compounds. Reaction of calf thymus DNA with *p*-benzoquinone produced three adducts as detected by ³²P-postlabeling. These adducts have been identified as (3'-hydroxy)-3,N⁴-benzetheno-2'-deoxycytidine-3'-phosphate; (3'-hydroxy)-1,N⁶-benzetheno-2'-deoxyadenosine-3'-phosphate; and (3'-hydroxy)-1,N²-benzetheno-2'-deoxyguanosine-3'-phosphate. The DNA adduct formed in HL-60 cells did not correspond to any of the principal adducts formed in DNA reacted with *p*-benzoquinone, suggesting that cellular environment modifies DNA adduct production by *p*-benzoquinone. These studies demonstrate that DNA adduct formation occurs in human bone marrow treated with benzene metabolites and suggest that PI-enhanced ³²P-postlabeling may be used to detect DNA adducts resulting from benzene exposure.

Introduction

Human exposure to benzene can occur from both occupational and environmental sources (1). In addition, a principal source of benzene exposure is cigarette smoke (2). Benzene is carcinogenic in rats and mice (3) and leukemogenic in highly exposed humans (4). Recent epidemiologic evidence also suggests that smoking is associated with an increased risk for nonlymphocytic leukemia (5). These results raise the question as to the risk associated with benzene-exposure and if measurement of benzene-DNA adducts can be used as a dosimeter of exposure.

Initial benzene metabolism occurs in the liver with formation of benzene oxide by P-450 oxidation followed by spontaneous conversion to phenol. This primary metabolite is further oxidized to hydroquinone (HQ) and catechol (6). These metabolites have been shown to accumulate in the bone marrow (7), and it has been postulated that myeloperoxidases (MPO) present in the myeloid bone marrow cells may convert HQ to *p*-benzoquinone (*p*BQ) (8).

The PI-nuclease-enhanced ³²P-postlabeling procedure has been shown to be a very sensitive method for the detection and quantitation of aromatic DNA adducts (9). In the present study we have used this procedure to measure DNA adduct formation in HL-60 cells and human bone marrow treated with the benzene metabolites *p*BQ and HQ.

Results and Discussion

Identification of Benzene-DNA Adducts

Reaction of *p*BQ with DNA produced three major DNA adducts as detected by ³²P-postlabeling (Fig. 1). Adduct 2 was the

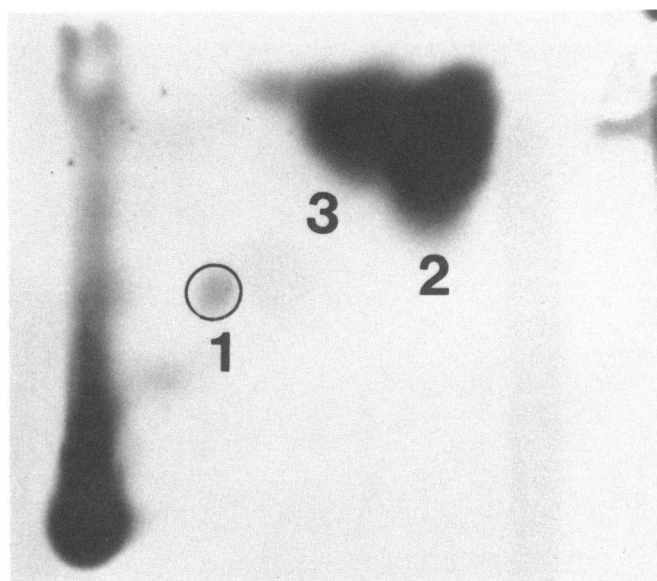


FIGURE 1. Autoradiogram of ³²P-postlabeled calf thymus DNA reacted with *p*-benzoquinone (*p*BQ). The reaction of *p*BQ with DNA, ³²P-postlabeling, and chromatography was performed as previously described (10).

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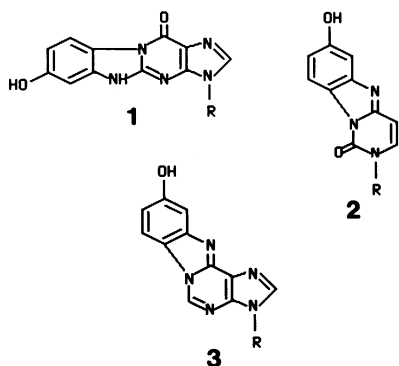


FIGURE 2. The structure of the DNA adducts formed by the reaction of *p*-benzoquinone with DNA (10–12).

principal adduct detected and accounted for 74.2% of the total modification. Adducts 3 and 1 accounted for 22.4% and 1.09%, respectively, of the modification. In order to identify the structures of these adducts, we carried out reactions of individual 2'-deoxyribonucleoside-3'-phosphates with *p*BQ and isolated the major products. Structural analysis and ^{32}P -postlabeling of the isolated products allowed identification of the DNA adducts as (3'-hydroxy)-3, N^4 -benzetheno-2'-deoxycytidine-3'-phosphate (adduct 2), (3'-hydroxy)-1, N^6 -benzetheno-2'-deoxyadenosine-3'-phosphate (adduct 3), and (3'-hydroxy)-1, N^2 -benzetheno-2'-deoxyguanosine-3'-phosphate (adduct 1) (10–12). The structures of these adducts are shown in Figure 2. The reaction mechanism leading to these cyclic products consists of initial reaction at the amino group followed by enolization and ring closure at the imino group.

Detection of DNA Adducts in HL-60 Cells

The majority of the myeloid bone marrow cells, namely, myeloblasts, promyelocytes, and immature granulocytes, have

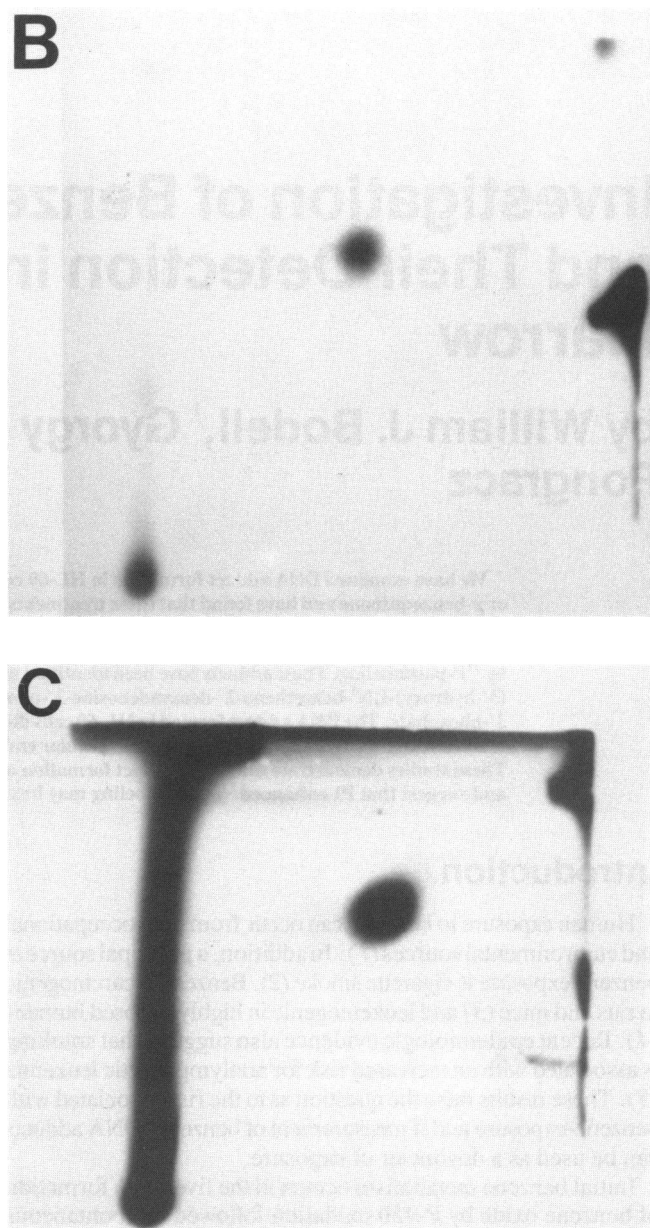
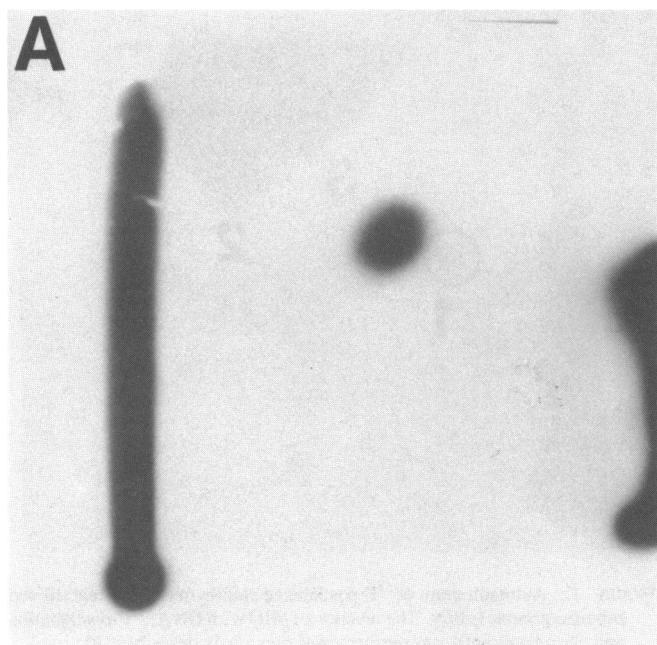


FIGURE 3. Autoradiograms of ^{32}P -postlabeled DNA. (A) DNA isolated from HL-60 cells treated with 100 μM of *p*-benzoquinone (*p*BQ) for 4 hr. (B) DNA isolated from HL-60 cells treated with 500 μM of hydroquinone (HQ) for 8 hr. (C) Co-chromatography of ^{32}P -postlabeled DNA from A and B. Treatment of HL-60 cells with *p*BQ and HQ and ^{32}P -postlabeling and chromatography was as previously described (12).

appreciable levels of MPO (13). As a model for DNA adduct formation in bone marrow, we used a human promyelocytic cell line (HL-60) that has significant MPO activity (14). Treatment of HL-60 cells with either 100 μM of *p*BQ for 4 hr or with 500 μM of HQ for 8 hr produced one DNA adduct as detected by ^{32}P -postlabeling (Fig. 3A and B). The relative DNA adduct levels resulting from these treatments were $10.9 \pm 1.0 \times 10^{-7}$ and $7.8 \pm 0.8 \times 10^{-7}$, respectively. Co-chromatography experiments established that the adduct produced by treatment of HL-60 cells with either *p*BQ or HQ was the same (Fig. 3C).

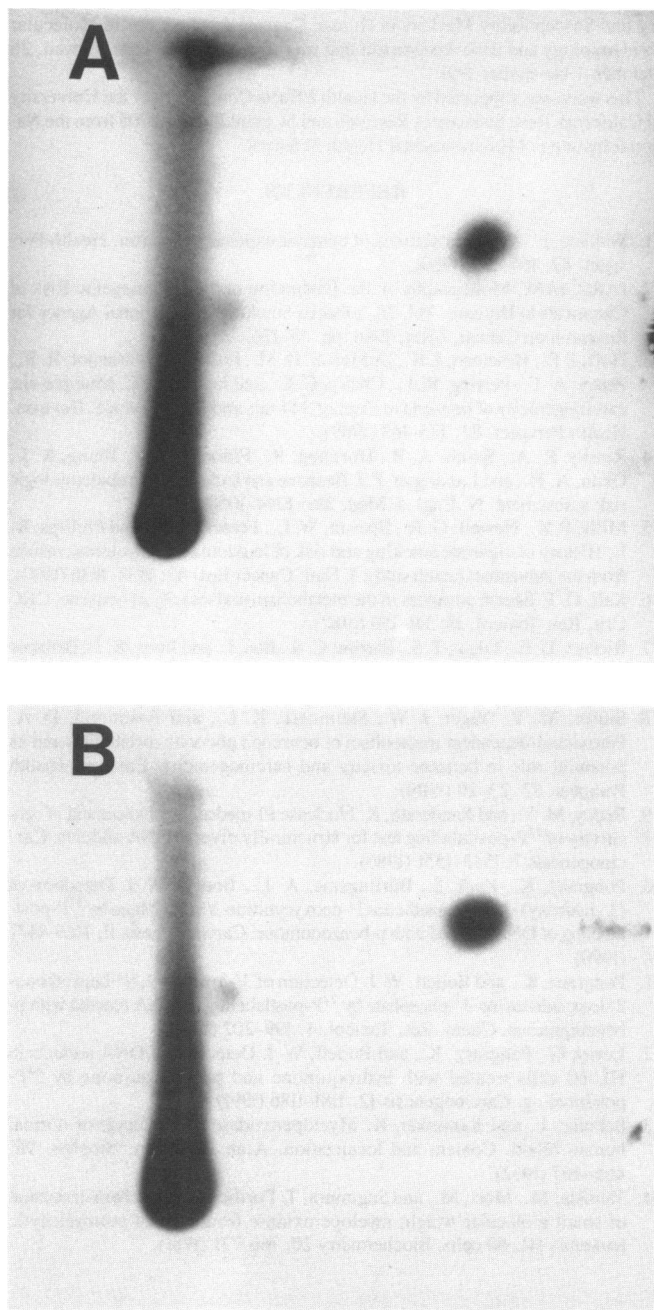


FIGURE 4. Autoradiogram of ^{32}P -postlabeled DNA. (A) DNA isolated from bone marrow treated with $250\ \mu\text{M}$ of *p*-benzoquinone (*p*BQ) for 2 hr. (B) Co-chromatography of ^{32}P -postlabeled DNA from A with ^{32}P -postlabeled DNA from HL-60 cells treated with $250\ \mu\text{M}$ of *p*BQ for 2 hr.

Detection of DNA Adducts in Human Bone Marrow

Bone marrow is the target tissue for leukemogenic effects benzene in humans. For our study we have obtained bone marrow samples from patients undergoing joint replacement operations. Treatment of bone marrow with $250\ \mu\text{M}$ of *p*BQ for 2 hr produced a single DNA adduct in bone marrow cells (Fig. 4A). Co-chromatography studies of ^{32}P -postlabeled DNA from bone

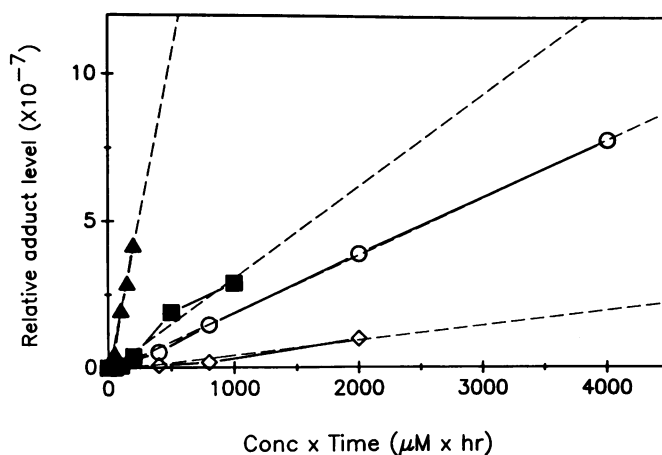


FIGURE 5. The induction of DNA adducts in HL-60 cells treated with *p*-benzoquinone (*p*BQ) (\blacktriangle) and hydroquinone (HQ) (\circ) and human bone marrow treated with various doses of *p*BQ (\blacksquare) and HQ (\diamond). The doses of each agent are expressed as the product of the concentration and treatment time. The slopes of these curves were estimated by linear regression analysis.

marrow treated with *p*BQ with ^{32}P -postlabeled DNA from HL-60 cells treated with $250\ \mu\text{M}$ of *p*BQ for 2 hr samples clearly show that the adduct formed in bone marrow cells is identical to that formed in HL-60 cells. (Fig. 4B).

Comparison of the extent of DNA adduct formation in HL-60 cells and bone marrow after HQ and *p*BQ treatment was made by analyzing the slopes of the dose-response curves (Fig. 5). For HQ treatment the slopes were 19.8×10^{-4} for HL-60 and 5.16×10^{-4} for bone marrow, whereas for *p*BQ treatment the slopes were 21.6×10^{-3} and 3.14×10^{-3} for HL-60 and bone marrow, respectively. Comparison of these values shows that HQ is approximately 4- to 5-fold and *p*BQ is approximately 7- to 8-fold more effective at adduct formation in HL-60 cells compared to bone marrow. A possible explanation for the reduced binding in bone marrow is that this tissue contains both erythroid and myeloid cells and only the myeloid cells have the capacity to activate HQ. These studies demonstrate that HL-60 cells are a good model for the activation and binding of benzene metabolites in the myeloid cells of human bone marrow.

Comparison of Adducts Formed in DNA and in Cells

DNA isolated from HL-60 cells treated with $250\ \mu\text{M}$ of HQ for 8 hr was ^{32}P -postlabeled and co-chromatographed with ^{32}P -postlabeled DNA reacted with *p*BQ. Figure 6 shows that the cellular adduct as indicated did not correspond to any of the major adducts formed in DNA.

Conclusion

The DNA adducts formed in HL-60 cells and bone marrow treated either with *p*BQ or with HQ are identical. These results are consistent with the requirement for HQ to be enzymatically oxidized to *p*BQ to form DNA adducts. The mechanism for activation of HQ is probably via MPO (8). The DNA adduct formed in HL-60 cells treated with either *p*BQ or HQ did not

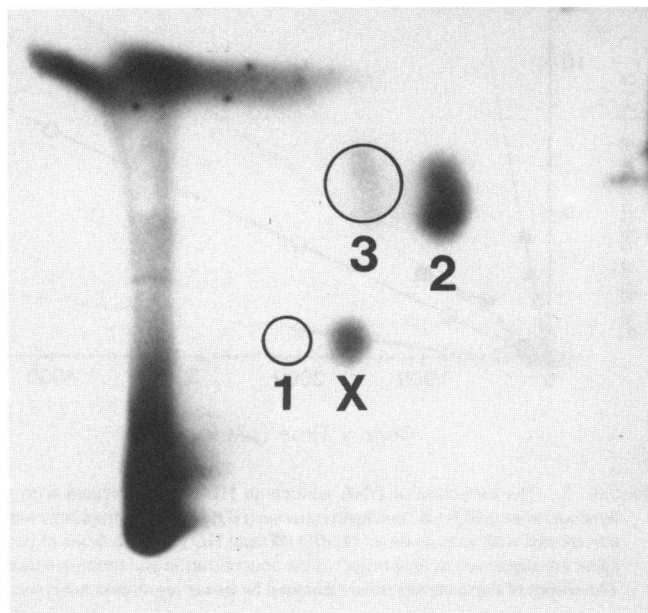


FIGURE 6. Autoradiogram of ^{32}P -postlabeled DNA. Co-chromatography of ^{32}P -postlabeled DNA from HL-60 cells treated with $250\ \mu\text{M}$ of hydroquinone for 8 hr with ^{32}P -postlabeled DNA reacted with *p*-benzoquinone. The DNA adducts from the *in vitro* reaction are indicated by numbers, and the cellular adduct is indicated by X.

correspond to any of the (3'-hydroxy)-benzetheno adducts formed in DNA reacted with *p*BQ. This result suggests that either chromatin structure or enzymatic processes within the cell are influencing DNA adduct formation by HQ and *p*BQ.

Our studies have identified a DNA adduct formed by the benzene metabolites hydroquinone and *p*BQ in human bone marrow and suggest that DNA adduct formation by these metabolites may be involved in benzene-induced leukemogenesis. The application of the PI-enhanced ^{32}P -postlabeling procedure should allow for detection of benzene-DNA adducts in populations exposed to benzene either occupationally or from tobacco smoke. However, these results also indicate that cells containing peroxidase activity such as neutrophils would be the appropriate cell type for monitoring the effects of benzene exposure, rather than lymphocytes, which do not contain peroxidase activity.

This manuscript was presented as a poster at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

This work was supported by the Health Effects Component of the University of California Toxic Substances Research and by grant P42ES04705 from the National Institute of Environmental Health Sciences.

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